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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

EARLY LYSIS, INDUCED BY CHLOROFORM, IN BACTERIA INFECTED BY BACTERIOPHAGE

Source - Annales de l'Institut Pasteur (Annals of the Pasteur Institute), Vol 90, Paris, 1956, pages 102-106.

Author - Janine Sechaud and E. Kellenberger

It is important to be able to study the intracellular development of bacteriophages before spontaneous lysis takes place (1). Different methods are known for the artificial inducement of lysis of infected bacteria: treatment via ultra sounds (2), external lysis via superinfection of the complexes with high multiplicities of another phage (3), explosion due to the sudden expansion of a highly compressed soluble gas (4,5), and treatment with concentrated amounts of glycin (6). We have observed that early lysis of complexes can also readily be obtained through the action of chloroform, from the instant that the bacteria contain at least one infectious particle. This property of chloroform was observed during experiments during which we were seeking to count the free phages present in a suspension, after elimination of the complexes by chloroform, according to the process indicated by Fredericq (7). In this article, we are presenting the result of several experiments showing the existence of an early lysis of the complexes, under the effect of chloroform.

Material and Techniques

We studied the multiplication of phages T2, T4, and λ after infection, respectively, by Escherichia coli B and Escherichia coli K12S, as well as after the induction of lysogenic E. coli K12 (λ).

The following media were used:

1. Tryptone medium: 1 percent Bacto-tryptone (Difco) and 0.5 percent NaCl.

2. Synthetic medium M9: 0.7 percent Na_2HPO_4; 0.3 percent KH_2PO_4;

0.05 percent NaCl; 0.1 percent NH_4Cl; 0.4 percent glucose; $CaCl_2$, 0.000 1 M; and $MgSO_4$, 0.001 M.

3. Synthetic medium M9 enriched with 1 percent amino acids (Difco).

4. Phosphatic plug: 0.7 percent Na_2HPO_4; 0.4 percent NaCl; 0.3 percent KH_2PO_4; and 0.02 percent $MgSO_4$.

The normal pH of these media is from 7.1 to 7.2.

The counting of bacteriophages was done through the classical techniques described by Adams (8).

Results

Phage Production Curves. Figure 1 represents the spontaneous production of phage (a) and the production observed after the action of chloroform (b-e) on the E. coli B - phage T4 complexes.

An aerated culture containing around 3.10^8 bacteria per ml is centrifuged, and the bacteria are again placed in suspension in an identical medium and then infected with proportions of phages ranging from 0.1 to 1.5 per bacterium. After 3 to 4 minutes of adsorption at 37° [Centigrade] with aeration, the suspensions are so diluted that one ml contains about 3.10^4 infected bacteria. At different times, samples are extracted and diluted in the selected pH plug containing 3-6 drops of chloroform per 5 ml. These suspensions are stirred vigorously for several seconds and, 15-30 minutes later, are spread over gelose in the presence of indicatory bacteria, in accordance with the technique of gelose in superfusion.

Curve e was obtained on the basis of a concentrated culture 8.10^8 infectious centers per ml in a synthetic medium enriched with amino acids. The treatment by chloroform was done on non-diluted samples; to reduce the readsorption, these samples were quickly cooled; we noticed, in effect, that the chloroform does not destroy the adsorption capacity of the bacteria.

The lysis induced by chloroform is independent of the medium used. The minimum latent period is from 21 to 22 minutes, and it is around the 15th minute that one can detect the presence of an average of one infectious particle per bacterium.

In the case of the bacteriophage T2, we obtained a higher number of plates in an alkaline medium (pH = 7.5) than an acid one (pH = 6.5). This result seems to be due not to a diminishing of the lytic effect of the chloroform but to an inactivation comparable to the one described by Sagik (10). Therefore it seems to us to be preferable, for the current experiments, to operate on a pH between 7.5 and 8.

Figure 2 shows the results of an experiment involving lysis induced by chloroform in E. coli K12 (λ) which is induced by ultraviolet ray (9). A culture containing around 3.10^8 bacteria per ml is diluted to 1/100 in plug, then exposed for 40 seconds, at a distance of 1 m, to the action of a WL 782 L Westinghouse sterilamp. In these condtions, the development of the phage is induced in 97 percent of the bacteria. The irradiated suspension is diluted in a tryptone medium so that 1 ml contains around 3.10^4 infectious centers. The treatment by chloroform is carried out in the same conditions as before. It is observed that the effect of the chloroform is independent of the pH. The minimum latent period for the development of the λ phage in the lysogenic bacteria induced is around 55 minutes, and it is around the 45th minute that there exists an average of one infectious particle per bacterium.

Kinetics of the Lysis Induced by Chloroform. It is important to know how rapidly the chloroform induces the lysis of the complexes. For that reason we agitated the complexes with the chloroform; then we set them out at different intervals, and after 2 minutes, the lysis appeared to be complete.

We also controlled the kinetics of the lysis by noting the visual density. We observed that 6 minutes after infection by T4, chloroform does not induce the lysis of the complexes. Added 12 minutes after infection, or later still, the chloroform induces a rapid lysis; within 10 minutes, the amount of optical density goes down to one tenth the initial amount. The measurements are made difficult by the presence of small drops of chloroform which remain in suspension for a relatively long time.

Inactivation of the Phages by Chloroform. We treated with chloroform fresh lysates of T3, T4, T5, and λ phages, the contents of which were known and rid of bacteria by centrifugation. A slight inactivation is notable, but it never exceeded 20 percent.

Discussion

The experiments that have just been taken up show that the treatment with chloroform is a very effective way of artifically inducing the lysis of phage-bacterium complexes, from the moment that the first infectious particles are formed.

The difference of about a minute that can be observed between Doermann's curves (3) and ours is probably attributable to small differences in the culture conditions (such as temperature, for example).

The lysis induced by chloroform turns out to be more rapid and simple than those obtained by the other known methods. However, its use is limited by the fact that it is utilizable only on bacterial populations already containing at least an average of one infectious particle per bacterium.

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FIGURE ARTICLE

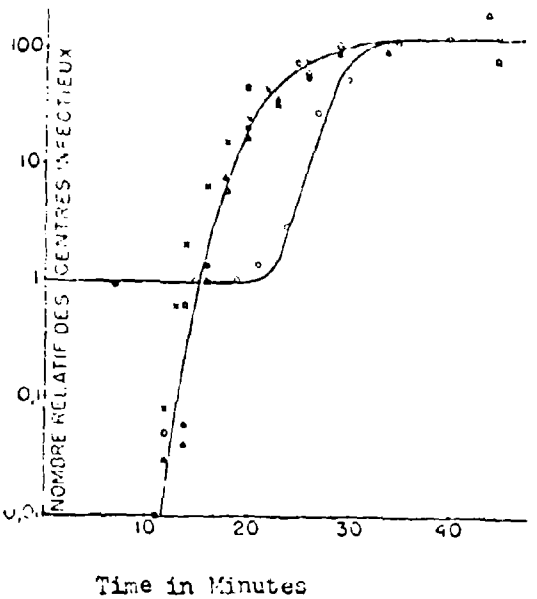

Figure 1. Intracellular appearance and liberation by spontaneous lysis of the T4 bacteriophage, multiplying itself on E. coli B.

- o (a) phages freed spontaneously (multiplicity of infection, m = 0.01)
- △ (b) phages freed by chloroform, at pH 8 (m = 0.01)
- ▲ (c) phages freed by chloroform, at pH 7.2 (m = 0.0^7)
- ● (d) phages freed by chloroform, at pH 6.6 (m = 0.01)
- □ (e) phages freed by chloroform, at pH 8, culture concentrated in amino medium (m = 1.5)
- × (f) phages freed by external lysis, according to the method of Doermann (3)